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PLATELET AGGREGATION IN THE END-STAGE RENAL DISEASE – DIFFERENCES BETWEEN PATIENTS TREATED WITH HEMODIALYSIS and PERITONEAL DIALYSIS

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End-stage renal disease patients (ESRD) suffer from procoagulant abnormalities that lead to excessive cardiovascular events, as well as from platelet dysfunction manifesting as an increased risk of bleeding. The exact pathogenesis of complex hemostatic disorders in ESRD patients is not completely understood. The aim of our study was to investigate the possible different effects of hemodialysis (HD) and peritoneal dialysis (PD) on platelet function in patients with ESRD by using the platelet function analyzer (PFA-100) which in vitro simulates the process of aggregation and platelet activation. Tests were performed with collagen/epinephrine (COL/EPI) and collagen/adenosine-5-diphosphate (COL/ADP) cartridges. The study included 44 patients with ESRD undergoing regular HD (n=32) or PD (n=12). Although there were no significant differences in COL/EPI and COL/ADP tests, it is indicative that more than 50% of HD patients had COL/EPI test values above the upper limit. These findings correlated with a higher chance for bleeding in HD group. Additionally, patients in HD group were significantly older and had significantly lower platelet count compared to PD patients.

Key words: platelet function, end-stage renal disease, hemodialysis, peritoneal dialysis

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INTRODUCTION

Hemostatic disorders are common complications in patients with end-stage renal disease (ESRD), mainly in the form of bleeding diathesis, but also as an increased risk of thrombotic events. In terms of abnormalities of primary hemostasis, platelet dysfunction and impaired interaction between platelets and vessel wall are considered as the main factors responsible for bleeding tendencies[1-3]. Anemia and accumulation of medication due to poor clearance are also important factors in impaired hemostasis in ESRD patients[1]. On the other hand, the high mortality rate in ESRD patients is mainly due to the increased incidence of thrombotic and cardiovascular complications despite decreased platelet function[3]. Hemostatic abnormalities in ESRD, to some extent, may be affected by the choice of renal replacement therapy[4,5].

Patients on hemodialysis (HD) have an increased risk of thrombotic events, primarily due to the release of thromboxane A2 and ADP into the circulation and also platelet degranulation. Some activation of platelets occurs due to the exposure of blood to the roller pump segment[2,3,5]. On the other hand, the hemodialysis process itself may contribute to hemorrhagic tendencies1, while uremic toxins present in circulating blood can be partially responsible for platelet dysfunction which can lead to bleeding diathesis. Patients on peritoneal dialysis (PD) showed evidence of a higher degree of hypercoagulation than HD patients[2,3]. The exact pathogenesis of complex hemostatic disorders in patients with ESRD is not completely understood.

The aim of our study was to investigate the possible different effects of HD and PD on platelet function in pa-

tients with ESRD by using the platelet function analyzer (PFA-100) which *in vitro* simulates the process of aggregation and platelet activation.

PATIENTS AND METHODS

The study included patients with ESRD on two different types of renal replacement therapy (HD and continuous ambulatory peritoneal dialysis, CAPD), treated at the Department of Nephrology, Hypertension, Dialysis and Transplantation, Zagreb University Hospital Center, during a 3-month period. On regular patient visit, together with blood sampling for standard laboratory parameters, an additional 2 mL of blood was obtained from all patients that met the inclusion criteria, after providing their informed consent. Ethical approval was obtained from the Ethics Board of the Zagreb University Hospital Center. Platelet function testing was performed on a platelet function analyzer (PFA-100) which *in vitro* simulates the process of aggregation and platelet activation. The tests were performed with collagen/epinephrine (COL/EPI) and collagen/adenosine-5-diphosphate (COL/ADP) cartridges. Results are reported as the closure times in seconds for COL/EPI (increased by aspirin and nonsteroidal anti-inflammatory drugs, NSAID) and COL/ADP cartridges (variably affected by ADP receptor disorders and clopidogrel). The ranges considered normal were 85-165 s for the COL/EPI closure time and 71-118 s for the COL/ADP. Data for analysis were taken from medical records.

PATIENTS

The study was performed on 44 ESRD patients undergoing regular HD or PD. The group of patients on HD included 32 patients (19 male and 13 female, median age 62), while the PD group included 12 patients (5 male and 7 female, median age 51). The cause of ESRD was hypertension in 12, diabetic nephropathy in seven, glomerulonephritis and pyelonephritis in nine, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP) in two and congenital diseases in seven patients, whereas in seven patients the cause of renal failure was unknown. The patients included in the study received all their regular medications except for drugs that affect platelet function (aspirin, anticoagulants, nonsteroidal anti-rheumatic drugs). Statins were used by two (6.3%) patients in HD group and 9 (75%) patients in PD group. In HD group, 24 (75%) patients received erythropoietin therapy, while in CAPD group only two (16.7%) patients received erythropoietin therapy. All but two patients in HD group were administered low-molecular-weight heparin (LMWH, nadroparin or enoxaparin). Patients in PD group did not receive LMWH. All patients in both groups signed their informed consent.

IN VITRO CLOSURE TIME TEST

The Platelet Function Analyzer (PFA-100, Siemens Healthcare Diagnostics) is a tool that can detect abnormalities of primary hemostasis in small blood samples. It measures the time required for the platelet plug from citrated blood aspirated under control flow conditions through a 150-micrometer aperture to occlude the aperture (closure time). The system monitors platelet interaction on collagen-ADP (COL-ADP) or collagen-epinephrine (COL-EPI) coated membranes[3,4]. In vitro closure time was measured according to the manufacturer's instructions using 800 μL of blood for each test (COL-EPI and COL-ADP).

Results are reported as closure times in seconds for COL/EPI (increased by aspirin and NSAID) and COL/ADP cartridges (variably affected by ADP receptor disorders and clopidogrel). The ranges considered normal were 85-165 s for the COL/EPI closure time and 71-118 s for the COL/ADP[6-9].

STATISTICAL METHODS

Statistical software IBM SPSS Statistics version 21 was used in all statistical procedures. Normality of data distribution was assessed with Kolmogorov-Smirnov test; based on these results and total sample size, appropriate nonparametric test was used in following analyses. Differences between HD and CAPD groups in categorical variables were analyzed with χ^2-test and differences in quantitative variables with Mann-Whitney U test. Spearman correlation coefficients were calculated between age, duration of treatment, COL/EPI and COL/ADP levels. All p values below 0.05 were considered significant.

RESULTS

Differences between HP and PD groups in categorical patient characteristics are shown in Table 1. There were no significant differences in gender, diagnosis, EPO (erythropoietin), statin, COL/EPI and COL/ADP groups, except for LMWH prevalence in HD group that was significantly higher compared to CAPD group ($p < 0.001$).
There were no significant differences in COL/EPI and COL/ADP tests (Fig. 1).
However, more than 50% of HD patients had COL/EPI levels higher than 165 s, i.e. delayed closure time.
Differences between HP and PD groups in quantitative patient characteristics.
Differences between HD and PD groups in quantitative patient characteristics are shown in Table 2. Significant differences were noted in age (HD group was significantly older; $p=0.025$) and platelet count (higher levels in PD group; $p=0.017$).

Table 1

Differences between HD and CAPD groups in categorical patient characteristics (χ^2-test)

		Group				p
		HD N=32		CAPD N=12		
		n	%	n	%	
Gender	Male	19	59.4	5	41.7	0.293
	Female	13	40.6	7	58.3	
Dg	Hypertension	9	28.1	3	25.0	0.796
	Diabetes	6	18.8	1	8.3	
	Glom/Pye	5	15.6	4	33.3	
	HUS/TTP	2	6.3	1	8.3	
	Congenital	5	15.6	1	8.3	
	Other	5	15.6	2	16.7	
EPO	No	8	25.0	2	16.7	0.557
	Yes	24	75.0	10	83.3	
LMWH	No	2	6.3	12	100.0	<0.001
	Yes	30	93.8	0	0.0	
Statin	No	30	93.8	9	75.0	0.081
	Yes	2	6.3	3	25.0	
COL/EPI	<85 s	2	6.3	1	8.3	0.245
	85-165 s	13	40.6	8	66.7	
	>165 s	17	53.1	3	25.0	
COL/ADP	<71s	4	12.5	0	0.0	0.294
	71-118 s	17	53.1	9	75.0	
	>118 s	11	34.4	3	25.0	

Figure 1
COL/EPI

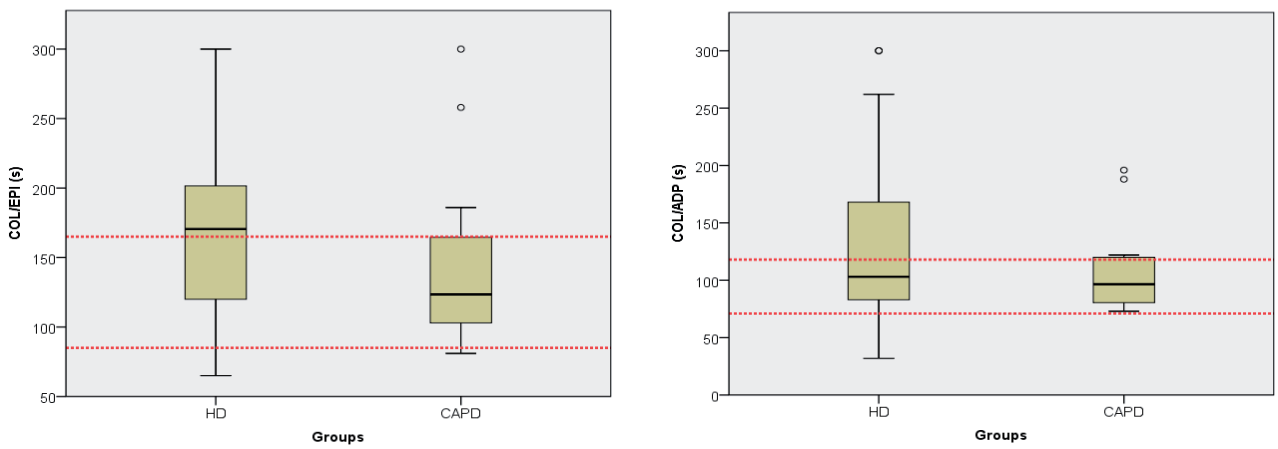

Table 2

Differences between HD and PD groups in quantitative patient characteristics (Mann-Whitney U test)

Group		n	Minimum	Maximum	Percentile 25th	Percentile 50th (Median)	Percentile 75th	p
Duration (months)	HD	32	6.00	126.00	17.25	36.50	65.00	0.094
	CAPD	12	4.00	144.00	9.50	16.00	35.75	
Age	HD	32	22.00	88.00	51.00	62.50	75.00	0.025
	CAPD	12	24.00	67.00	43.50	51.00	60.50	
TT	HD	31	41.00	94.00	56.00	64.00	71.00	0.432
	CAPD	12	42.00	95.00	54.75	68.50	75.75	
TV	HD	31	153.00	191.00	162.00	168.00	175.00	0.989
	CAPD	11	153.00	187.00	158.00	168.00	173.00	
E	HD	32	2.72	4.24	3.22	3.54	3.77	0.370
	CAPD	12	2.26	5.15	3.40	3.66	3.84	
Hgb	HD	32	76.00	127.00	104.50	111.50	119.00	0.989
	CAPD	12	87.00	139.00	97.00	113.50	118.50	
Plt	HD	32	111.00	413.00	149.00	173.00	209.00	0.017
	CAPD	12	150.00	302.00	186.75	226.50	265.00	
MPV	HD	32	6.70	12.40	7.90	8.50	9.10	0.926
	CAPD	12	6.30	9.70	7.58	8.80	9.25	
L	HD	32	2.70	12.80	5.03	6.05	7.63	0.823
	CAPD	12	3.50	12.60	4.63	5.60	7.68	
COL/EPI (s)	HD	32	65.00	300.00	119.50	170.50	201.75	0.215
	CAPD	12	81.00	300.00	100.00	123.50	175.25	
COL/ADP (s)	HD	32	32.00	300.00	81.00	103.00	171.00	0.580
	CAPD	12	73.00	196.00	80.25	96.50	121.00	

DISCUSSION

Previous studies confirmed the existence of dysfunction of primary hemostasis in patients with ESRD measured by PFA-100, a platelet function analyzer, and the ability of HD to correct some part of hemostatic disturbances[5,10]. It was also found that 60% of dialysis patients had prolonged in vitro closure time measured by PFA[10]. In our study, there was no statistical difference in platelet function measured by using the platelet function analyzer (PFA-100) and expressed as in vitro closure time between HD and PD patient groups. However, it is indicative that more than 50% of HD patients had COL/EPI test values above the upper limit. These findings could be clinically correlated with a higher chance for prolonged bleeding in HD patients. The number of patients included in the study in both groups was relatively small and this could be the possible reason for statistically nonsignificant difference in platelet function expressed as closure time. Furthermore, these findings could be correlated to the fact that hemodialysis patients received LMWH, which was not administered to PD patients. It was already observed in a clinical study that platelet dysfunction existed in approximately half of the chronic HD patients and did not improve after HD, regardless of the anticoagulation regimen used[11].

In our study, we also found a statistically significantly lower level of platelets in HD group compared to PD (p=0.017), although platelet counts were within the reference range in both groups. It is consistent with previous observations from different clinical studies, which showed that in predialysis patients, as well as in HD patients, platelet count tended to be reduced1 in the range of 175.000-180.000/mm^3 compared with 250.000/mm^3 in healthy controls. In PD patients, platelet counts have been reported to be closer to the normal range[12-14].

Another study that compared the effects of dialysis with four different heparin protocols, using either unfractionated heparin or LMWH in two different dosages, showed that decreases in platelet counts were similar

with all anticoagulation regimens used[12,15,16]. Besides the statistically significantly lower platelet level in HD group, patients in this group were also older compared to CAPD group.

CONCLUSION

In the present study, we found no statistical difference in platelet function measured by the platelet function analyzer (PFA-100) and expressed as in vitro closure time between HD and PD patient groups, but it is indicative that more than 50% of HD patients had COL/EPI test values above the upper limit, supporting the possibility of clinical correlation with a higher chance for increased or prolonged bleeding in HD patients. According to the results of this study, we can conclude that PD as a method of renal replacement therapy showed better safety profile concerning platelet function, with less reduction in platelet count and no need for LMWH. The sensitivity of the test and clinical relevance of these findings should be further investigated in a larger study with more patients included.

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SAŽETAK

AGREGACIJA TROMBOCITA U ZAVRŠNOM STADIJU ZATAJIVANJA BUBREGA – RAZLIKE IZMEĐU BOLESNIKA KOJI SU LIJEČENI HEMODIJALIZOM I PERITONEJSKOM DIJALIZOM

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Završni stadij kronične bubrežne bolesti obilježen je različitim prokoagulantnim odstupanjima koja dovode do razvoja tromboembolijskih komplikacija uz istodobno poremećenu funkciju trombocita s posljedičnim porastom rizika za nastanak krvarenja. Točna etiologija složenih hemostatskih poremećaja u završnom stadiju kronične bubrežne bolesti nije u potpunosti razjašnjena. Cilj ovoga istraživanja bio je usporediti učinak hemodijalize i peritonejske dijalize na funkciju tromobocita kod bolesnika u završnom stadiju kronične bubrežne bolesti primjenom analizatora funkcije trombocita (PFA-100) koji in vitro stimulira proces aktivacije i agregacije trombocita. Ispitivanje je provedeno na 2 testa (COL/EPI i COL/ADP) koji mjere vrijeme potrebno cirkulirajućoj krvi da okludira membranu obloženu kolagenom i adrenalinom (COL/EPI) odnosno kolagenom i ADP-om (COL/ADP). U istraživanje su bili uključeni bolesnici na hemodijalizi (n=32) odnosno peritonejskoj dijalizi (n=12). Premda nije zabilježena statistički značajna razlika između testova COL/EPI i COL/ADP, indikativno je da su u više od 50% ispitanika na hemodijalizi vrijednosti testa COL/EPI bile iznad gornje granice referentnog intervala. Ovi rezultati mogu se povezati s većom mogućnosti krvarenja u bolesnika na hemodijalizi. Uz to, bolesnici na hemodijalizi bili su značajno stariji te su imali statistički značajno niži broj trombocita u odnosu na ispitanike na peritonejskoj dijalizi.

Ključne riječi: funkcija trombocita, završni stadij kronične bubrežne bolesti, hemodijaliza, peritonejska dijaliza